PERSPECTIVES

Tadpoles, froglets, and conservation: A discussion of basic principles of rearing and release procedures

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Abstract.—We outline component features of the captive environment and the natural world that should be considered when designing a program for head-starting and releasing amphibians, presumably as part of a conservation project. The main points indicate the importance of accounting for features of the basic biology of amphibian larvae, the biology of the focal species, and highlight the types of error risks based on generalities, human convenience, and logistical limitations. Similarly, we urge consideration and evaluation of the quality of the metamorphs that are produced over the sheer quantity produced and released. While most of the examples are taken from pond-breeding species, the general principles are relevant, and details may be modified to fit amphibian species with larvae in other habitats.

Key words. Amphibians, conservation, larvae, reintroductions, translocations, head-start, captive breeding

Captive rearing of endangered frogs is a large, expensive (Mattioli et al. 2006), and time-consuming component of conservation efforts. We discuss a number of factors intended to improve the chances of successful rearing and release of captive individuals (McGregor and Zippel 2008). Central themes are 1) acceptance that captive-reared animals differ in a number of ways from those from natural populations (e.g., Griffiths and Pavajeau 2008; Gawor et al. 2012); 2) rearing protocols must address the specific biology of each species (e.g., Morrison and Hero 2003); 3) avoidance or reduction of activities that cater only to our conveniences; and 4) evaluation of criteria to judge success based on metrics other than just numbers released. We emphasize the need for long-term monitoring of the success of the releases. Most suggestions center around testing, improving, and standardizing species-specific procedures once those that produce individuals of the highest probable fitness are verified, not just the most individuals. Tadpole mortality varies across experimental venues (Melvin and Houlahan 2012), so survival and fitness likely vary according to husbandry regime, release protocols, and even captive breeding itself.

We argue that each taxon-specific system should directly address several types of questions. Do artificial environments and particularly the food sources used in captive programs alter reared froglets relative to what wild individuals experience (i.e., rapid acclimation to captivity of Griffiths and Pavajeau 2008)? Do these alterations adversely manifest themselves in the survival and fitness of 1) the released animals; 2) the population in which the animals are released; or 3) the meta-

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populations with which they interact? The real question is: do the introductions contribute in a beneficial way to conservation, or is there the appearance of conservation (through numbers) when actions actually degrade the fitness of the population (e.g., via artificial selection)? How do the morphological, immunological (Venesky et al. 2012), and hematological (Davis and Maerz 2011; Davis 2012) conditions of reared froglets compare to those of wild individuals? For example, Burns et al. (2009) found that the first-generation of captive-bred and reared guppies had smaller brains than wild individuals, and Fraser (2008) noted that re-introduced leopard frogs showed abnormal behaviors in the wild. It must be recognized that captive breeding, often with limited choices of mates and usually with small numbers of individuals, constrains or eliminates the possible beneficial effects of sexual selection (Williams and Hoffman 2009). Captive rearing of larvae from the wild may avoid problems related to sexual selection, but nonetheless impose selective pressures on the offspring. Because fisheries researchers have faced these problems for years, they have a good perspective based on the pitfalls of releasing captive-reared individuals into the wild. Studies from fisheries science have indicated that the quality or fitness of captive-reared individuals are not necessarily equivalent to those of wild individuals (e.g., Araki et al. 2007; Christie et al. 2012). Amphibian conservationists would be advised to review the references cited herein and create means of measuring and assessing the biological quality of wild and captive tadpoles.

Within these contexts, we recognize the limitations of funding, the expectations of administrators, and the expertise of personnel sometimes can work against the success of a given program. Stakeholders and participants in conservation programs may be pressured to, or evaluated on, the release of as many individuals as possible, as soon as possible, with minimal cost. These realities can lead to the implementation of inordinate or biologically inappropriate actions that may not increase the chance of the timely release of more viable individuals. For example, we have heard comments such as "*I do not understand. Some of them took off swimming across the pond and some of them got balled up in sand like they did not know what was going on.*" In fact, reared individuals may not perform successfully at basic tasks like prey recognition or seeking refuge, but they can be given a chance to learn (i.e., pre-release training of Griffiths and Pavajeau 2008, or the "soft-release" concept as it is termed in some conservation programs). Other statements that we have overheard include "*That skinny tadpole likes to lie on its side,*" "*I do not know why that tadpole swims in a spiral,*" "*These tadpoles constantly swim up and down the wall of the aquarium.*" All of these cases describe tadpoles that either are sick, stressed, or otherwise unsuitable for release, and examples of at least the first two cases should certainly be culled to improve the collective quality of the cohort (e.g., Nye and Cameron 2005).

We looked at two kinds of relevant information from the natural world to get a relative idea of what is faced in terms of the odds of survival in amphibian breeding. This perspective is crucial for evaluating captive programs, but seems to be poorly considered by practitioners. Some stakeholders we have observed seem to evaluate programmatic success by trying to maximize the number of metamorphic individuals that are released. Survival rates from egg to metamorphosis range from 0–20 % and are commonly 1–5 % (Wells 2007: table 14.5, fig 14.9). Survival from metamorphosis to first reproduction ranges from 6–26% (Herreid and Kinney 1966; Licht 1974). Greenberg and Tanner (2005) tracked the success of 23 breeding events of *Scaphiopus holbrookii* at eight sites in Florida over nine years; five of these events were considered successful by producing a minimum of 100 metamorphs likely derived from > 107 eggs deposited on site. Semlitsch et al. (1996) reported only one reproductive event at one site that produced significant numbers of metamorphs of *S. holbrookii* over 16 years. High levels of mortality are typical of many amphibian reproduction efforts, and efforts of husbandry to avoid such mortality may not be desirable.

Survival and fitness are correlated with environmental conditions and diet. Dietary requirements of metamorphs may be easily underestimated. For example, a grand cohort of 7,000 (1,000 each of seven species) metamorphs can consume at least 2.3×10^6 insects the size of *Drosophila* in the first post-metamorphic month (JRM and RA, unpubl. data). This number, about 2,250 g, will produce about 930 g of frog tissue (RA, unpubl. data). A frog needs about 20 cal/day/g body weight for maintenance at 20 °C (Mazur 1968). At 5796.6 cal/g dry weight of fly tissue (Cummins and Wuycheck 1971), one can calculate that a frog could consume about 2.2–4.2 times the calories needed for maintenance during the first post-metamorphic month. Also, it must be recognized that specific conditions at one point in the rearing process can influence the quality of an individual much later in ontogeny (e.g., Scott et al. 2007; Gervasi and Foufopoulos 2008; Gagliano and McCormick 2009; Uller et al. 2009; Van Allen et al. 2010) or perhaps more importantly, in subsequent generations (Frost et al. 2010). Because the behavioral, immunological, morphological, and physiological qualities of reared individuals seldom are measured or evaluated in amphibian programs, there is little idea if techniques (e.g., diet) are producing individuals of good quality. There are many factors that modify various qualities of metamorphs and postmetamorphs (e.g., Alford 1986; Blouin 1991, 1992; Gramapurohit et al. 2004; Relyea and Auld 2005, and many others). In light of these data, the percentage of individuals produced are likely quite a poor estimator of success after release, and success should not be equated with the introduction of the largest possible number of tadpoles or metamorphs into the wild.

The Natural World

We emphasize that not all aquatic larvae are adapted to similar aquatic environments. For example, phytotelmata are very different environments than are streams or ponds. Yet, the default rearing vessel for amphibians in captive programs is a stock, straight-walled aquarium. Focusing on the natural pond, for this example, let us consider this is an environment with a large surface area per volume for gas exchange and waste dispersion, wind and temperature induce water movements, and populations of plants and animals dispense with metabolites. The sloping banks allow tadpoles to escape some predators and seek water of the desired temperatures during development, and those approaching metamorphosis can safely switch from gas exchange across the gills to the lungs. During their daily movements about the pond, tadpoles learn directions to proper areas involving temperature, food, and refuge. The default enclosure with vertical walls and uniform depth violates all of these natural conditions, and the larvae have no exposure to differing microclimates, a natural light cycle, or the myriad ecological conditions that wild individuals encounter. Careful consideration of the egg deposition sites chosen by breeding frogs, and post-hatching behaviors of tadpoles should inform all aspects of the rearing enclosure, its placement, and environment. We should consider whether it is even possible to raise high-quality frogs indoors, or tropical frogs in a typical Nearctic zoo?

With respect to the natural world, and returning to the idea of pre-release training (Griffiths and Pavajeau 2008), we suggest that survival after release would increase appreciably if the simple tenets of Y-axis orientation (Ferguson et al. 1965; Taylor and Ferguson 1969) were implemented. This type of orientation allows individuals to move about their habitat productively as they receive input visually or via the pineal complex (i.e., nonvisual, including the patterns of light polarization; Taylor and Adler 1970). An accurate sense of time is involved, and the biological clock must be reset each day by witnessing sunrise in order to stay synchronized with changes in day lengths. A fixed light-dark cycle in the laboratory does not entrain the animals in any way because there is no appropriate movement of the "sun" and no changes in day length. Naive tadpoles released without training may have a higher probability of being predated, and a released metamorph (i.e., small size with poor locomotor skills, large surface-volume ratio and thus rapid water loss, likely with small energy reserves, and no idea of the locations of proper refuges) that makes one wrong directional choice has a high probability of dying.

We advocate that the adoption of the research protocol of Taylor and Ferguson (1969) into the release procedures would surely improve the success of the project. All that is needed to follow our pond-breeding example is: construct a meshed, wire cage with a top and bottom (about $100 \times 50 \times 30$ cm placed with its long axis about a third on land and two-thirds in the water, place tadpoles or metamorphs in the water and include moist cover for froglets, and wait for at least two days). Tadpoles and froglets of stream-breeding species (Preininger et al. 2012) obviously would also benefit from this procedure with some innovation of different meshed enclosures for stream species. The point is that, by experiencing even two sunrises, the animals will know the Y-axis, and when they are released, the animals can be expected to have a much better chance at survival because they are more likely to make the appropriate decisions.

The Culture World

We comment on several related topics that we feel are important; in all cases, stage refers to Gosner's (1960) table. The various forms of egg clutches (Altig and McDiarmid 2007) may be quite fragile, but individual eggs are much more robust than one would assume. Clutches can be pulled or cut apart without damaging the ova to improve the surface area/volume of the groups. In the case of pond breeding species, eggs should be placed in water not much deeper than the groups of eggs. Different protocols are advisable, of course, for species that breed in substantially different environments (e.g, streams or phytotelmata). The natural history of the species and the specific conditions under which its larvae develop must be considered and incorporated into the rearing protocols.

With respect to tadpoles, consider those of the Costa Rican Leaf Frog *Agalychnis lemur* that occur in very shallow, virtually non-moving swamps in nature; this must be considered when rearing this species in the lab. Many zoos and labs rely on stock aquaria, or similarly shaped tanks, that often are poor choices for rearing containers. They have a small surface area/volume, and this is a problem exacerbated by the tendency to put too many individuals in a given tank. If a caretaker laments the management of air stones and water changes, then the system is incorrect by definition. Information on management of water quality are reviewed in Poole and Grow (2012) and Pessier and Mendelson (2010). Tadpoles that swim up and down the glass are signaling that they are stressed by inappropriate temperatures, oxygen concentrations, or lack of naturalistic gradients of these crucial variables. Patterns of temperature variation can unpredictably influence developmental rate and morphology (Arrighi et al. 2013). Similarly, inappropriate quantities of food or refugia, waste buildup, or population density will also cause stress in tadpoles. As an example of our concept, consider a hypothetical pond-breeding species. Shallow pans are not recommended. They have a reasonable surface area/volume, but their total volume is small and thus water chemistry is quickly overwhelmed by food and feces, and catastrophic water loss to evaporation is easy to miss. A plastic wading pool or some similarly shaped, shallow enclosure is the best

because of the large surface area/volume. Aeration is not needed and water changes will only be necessary 1–2 times during the rearing of a batch of 300 tadpoles in a 143-cm diameter pool. Water depth in these pools does not need to exceed 6–8 cm and usually 4 cm is sufficient for most pond-breeding species; it is the surface area that is important because deeper water does not increase the usable space for more tadpoles. Flow-through systems decrease the manual work involved but are probably the worst at providing the animals the chance to acquire the proper intestinal bacteria.

We contend that the fear of the chytrid fungus, other pathogens, and caretakers' zeal for cleanliness has stimulated keepers to create overly clean environments, and this action deprives the tadpoles of acquiring intestinal symbiosis (Pryor and Bjorndal 2005a,b). Coprophagy, especially after the material has developed large populations of microbes and protozoan, is a viable feeding tactic of tadpoles (Steinwascher 1978). Careful management of diseases in captivity is based more on common-sense husbandry protocols, rather than absolute sterilization (Pessier and Mendelson 2010). Proper work-flow regimes, dedicated equipment, and vigilant monitoring of water quality are recommended over routine water-changes (in the absence of measurements to validate such) and bleaching of tanks and substrates.

Tadpoles and metamorphs have been fed many different foods (e.g., Modzelewski and Culley 1974; Claussen and Layne 1983; Jörgensen 1989; Pryor 2003; Hailey et al. 2007; Pramuk and Gagliardo 2008; and many others), but decisions and choices of foods do not appear to be based on data derived specifically from developmental performances, size, or energetic reserves present at metamorphosis. Amphibians sequester most of their body fat in the fat bodies attached to the anterior ends of the kidneys. Assessing the size of the fat body (i.e., dissection of a few specimens, or perhaps development of techniques using sonography or electrical conductivity; Walsberg 1988) of individuals raised on different diets would be a valuable endeavor. Part of the problem is that caretakers are just starting to distinguish between the consummatory and digestive diets of tadpole, with the real distinction being between what tadpoles ingest versus what they digest (Altig et al. 2007; Schiesari et al. 2009; Whiles et al. 2009). How oral structures influence feeding performances (e.g., Venesky et al. 2010a,b) also remains to be determined. The main point is that tadpoles swallow large quantities of the products of primary productivity (e.g., plants or algae), but when one considers periphyton, it is likely that these plants or algae may not represent the primary energetic intake (Altig et al. 2007). For example, one might consider adding a bit of clay soil to the rearing containers (Hailey et al. 2007; Akers et al. 2008). Adding supplements of natural foods, such as wild-collected aquatic plants or algae, is a viable idea, but one can inadvertently add vicious predators as well as potential pathogens and parasites. Algae can be allowed to develop in tanks, and there are protocols for cleaning plant materials before introduction to tanks (Pessier and Mendelson 2010); in either case, the important periphyton will develop. Tetramin® fish foods (TetraWerke, Melle, Germany), which contain considerable amounts of animal-based material, and powdered rabbit pellets have been used successfully by the authors, but there are no data on actual developmental performances. There are now many recipes for tadpole diets applied to a number of different programs for ranids (Abrahamse and Hayes 2009) and other taxa. These kinds of feed promote rapid production of microorganisms which likely serve as the primary food source for tadpoles. The point to keep in mind is that the knowledge-base for tadpole diets in the wild and nutritional needs is poor and far from taxonomically complete. Basic research in the form of controlled studies is necessary for virtually all species.

If the program is designed to release post-metamorphic individuals and rearing conditions have been suitable, then the majority of tadpoles will reach metamorphic stages 41–42 (i.e., eruption of front legs) at nearly the same time (Wells 2007). If a large proportion (e.g., 75–90 %) of the tadpoles do not metamorphose over a short period of time, then one may assume an excessive population density or some other factor has impeded normal growth. Metamorphs should be held until tail resorption is complete because tailed individuals have reduced locomotor abilities. If post-metamorphic frogs are to be maintained in captivity, then abundant and diverse small prey must be available. A single-species diet of fruit flies alone does not match the diversity of nutrients available to free-ranging froglets.

Release of Reared Individuals

After considering the release options outlined above, the release of tadpoles and froglets should be coordinated with when metamorphosis of the target species occurs at the release locality. This detail will increase the chances of there being proper weather conditions and sufficient food available, and one might consider verifying the local prey base (Goldstein 2007). Iterative assessments, via monitoring, and appropriate modifications of the release environment may be required. In the real context of the interactions of biotic and abiotic conditions, local populations increase and decrease through time. Populations in "good localities" (sources) persist for long periods, and populations in sites of some unknown lesser conditions (sinks) appear and disappear abruptly on short time scales. If a population did not succeed at the target site under the natural conditions, one should question the logic of a restoration attempt at that site; at least we should understand the reasons for its original failure. Also, the concept of source-versus-sink populations presents a difficult decision if one wishes to establish a population at an entirely new site (e.g., Pellitteri-Rosa et al. 2008; Mc-Murry et al. 2009; Ruiz et al. 2010). In any case, popu-

lations at sink-sites may sometimes represent important, if ephemeral, connectivity across the meta-population landscape. It is quite unlikely that any two adjacent pools present the same conditions, and in such a case, one must set aside human notions and conveniences, know the biology of the target species very well, evaluate the new site in detail, and diversify as much as is feasible with the number of individuals available. Various factors that change as a site undergoes succession can also change the likelihood of a given site being a viable site for reintroduction. Also, released individuals likely perform differently relative to other local taxa (Tingley et al. 2011).

Although there can be repercussions in doing so, one might consider reducing the chance of predation. Snakes can decimate tadpoles and froglets, but predatory fishes usually are not a problem in ephemeral sites, streams, or other types of sites where many frogs breed. Examples of the types of things to be considered for pond sites include comparisons of sites with no aquatic vegetation or short, sparse vegetation; stands of tall vegetation (e.g., especially cattails), zones covered by water lilies, and dense stands of emergent vegetation are not acceptable. These vegetation structures provide excessive organic debris that can reduce oxygen concentrations and excessive shade that inhibits proper periphyton growth. Dense stands of filamentous algae and algal mats are not acceptable because these populations reduce the oxygen concentration and some of these organisms are toxic. All manners of emergent, submergent, and floating vegetation must be considered with direct respect to the anticipated micro-habitat use of the released species.

One should also consider the qualities of adjacent terrestrial areas. High densities of froglets can occur at release sites, so additional refuges ought to be provided if one suspects that refugia could possibly be a limited resource. Artificial refugia made from PVC pipe (narrow gauge; cap on the bottom and a T-cap at the top; small drain hole about two cm from bottom to avoid filling with water; painted black) placed upright in the local environment will be used by post-metamorphic treefrogs (RA, unpubl. data). Pushing a rod into soft soil at a low angle and removing it leaves preliminary burrows for toads and ranid frogs, and pieces of PVC pipe laid on the ground and covered with soil to avoid overheating provides similar burrows. At the same time, this technique is subject to invasion by introduced fire ants (*Solenopsis invicta*) in southeastern North America; the ants use the tubes to help establish a mound and consume any froglets that may enter. Untreated wooden cover boards also can be quite useful as retreats if there is enough local moisture.

As an alternative to rearing tadpoles in pools in the laboratory, and the various concerns raised here above, it may be preferable to simply transfer eggs to the new site. Breaking a clutch into smaller pieces to enhance aeration would be prudent because the eggs likely are not placed in the same manner (e.g., attached to a twig off the bottom) as was done by the ovipositing frogs. Protection from egg predators (e.g., mesh enclosures) would be advisable. Egg transfers between already inhabited sites can facilitate genetic connectivity between sites, if that is what the management plan recommends. The program should not reduce genetic diversity. Reintroduction to a new or extirpated site may need multiple releases, not only for establishment, but for genetic management (e.g., if only F_1's from captive adults were released, and there is no connectivity to other populations, there would be inbreeding depression, genetic drift, etc.). Long-term genetic maintenance should be considered when deciding where and how often animals are released.

Conclusions

The study of amphibian declines is difficult, and the search for solutions is frustrating (e.g., Beilby et al. 2009). When release programs either succeed or fail, we often are never sure of the reasons why in either case, and volumes of anecdotal information are produced. Knowledge of the genetic diversity of the populations that are released (e.g., Charmantier and Garant 2005) is crucial, and throughout our rearing attempts, we must be certain that caretakers are not perpetuating any initial problems (e.g., Walker et al. 2008). We understand that some of the points we have raised may violate restrictions of funds, personnel, facilities, and time. But, we suggest that the bar should be raised at every available chance. If implemented at the design-phase of a conservation project, our recommendations require fewer resources than traditional programs so long as the crucial component of long-term post-release monitoring is equivalent. More field data on the biology of the species involved are needed, and many of the practical or financial limitations can be overcome by rather minor changes in techniques based on better knowledge of species biology. No protocol will ever approach total success, especially when details of why the targets met their demise in the first place. Some researchers who have made multiyear releases of head-started frogs at a site, but have not yet started routine monitoring seem uninformed. Perhaps the biggest idea in this discussion is that it must be remembered that imposing non-natural conditions (Gawor et al. 2012) on tadpoles and froglets by the seemingly simple act of culturing these organisms (Denver and Middlemis-Maher 2010) should underscore all aspects of the design and evaluation of a conservation program. The quality of the released individuals, the release protocol, and post-release monitoring are the most important factors to reconsider in any amphibian reintroduction or relocation program.

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